Current Biology 18, 1495–1500, October 14, 2008 ©2008 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.08.050

Report

Aluminum-Dependent Root-Growth Inhibition in *Arabidopsis* Results from AtATR-Regulated Cell-Cycle Arrest

Megan A. Rounds[1] and Paul B. Larsen[1,*]
[1]Department of Biochemistry
University of California
Riverside, California 92521

Summary

Aluminum (Al) toxicity is a global problem severely limiting agricultural productivity in acid-soil regions comprising upwards of 50% of the world's arable land [1, 2]. Although Al-exclusion mechanisms have been intensively studied [3–9], little is known about tolerance to internalized Al, which is predicted to be mechanistically complex because of the plethora of predicted cellular targets for Al^{3+} [2, 10]. An *Arabidopsis* mutant with Al hypersensitivity, *als3-1*, was found to represent a lesion in a phloem and root-tip-localized factor similar to the bacterial ABC transporter ybbm, with ALS3 likely responsible for Al transfer from roots to less-sensitive tissues [10–12]. To identify mutations that enhance mechanisms of Al resistance or tolerance, a suppressor screen for mutants that mask the Al hypersensitivity of *als3-1* was performed [13]. Two allelic suppressors conferring increased Al tolerance were found to represent dominant-negative mutations in a factor required for monitoring DNA integrity, *AtATR* [14–17]. From this work, Al-dependent root-growth inhibition primarily arises from DNA damage coupled with AtATR-controlled blockage of cell-cycle progression and terminal differentiation because of loss of the root-quiescent center, with mutations that prevent response to this damage resulting in quiescent-center maintenance and sustained vigorous growth in an Al-toxic environment.

Results and Discussion

Based on previous results from our *als3-1* suppressor screen [13], three strong suppressor lines were chosen for further study, with results indicating that the increase in growth in the presence of Al seen for these results from enhanced Al tolerance, because indicators of Al damage and response are induced normally in these mutants compared to WT. All three of the suppressor mutants that were originally studied represented dominant mutations that were localized to the same region of the *Arabidopsis* genome on the top arm of chromosome 5. One of these mutants, *alt1-1*, was chosen for a map-based cloning exercise in order to isolate the mutation of interest (Figure 1A). Sequencing of candidate genes found in the mapping window revealed that *alt1-1* represents a mutation resulting in the substitution of A for G in *At5g40820*, which encodes the previously characterized AtATR (ataxia telangiectasia-mutated and Rad3-related) [14], homologs of which are required for assessment of and response to DNA damage in all higher eukaryotes. In *Arabidopsis*, ATR functions to detect single-stranded breaks and replication fork blocks, with *atr* loss-of-function mutants being hypersensitive to agents that trigger these types of DNA damage [14–16]. The *alt1-1* mutation results in the change of Gly to Glu at position 1098, which is part of the uncharacterized UME (UVSB PI3K, MEI-41, ESR1) domain found in all reported ATR homologs that is predicted to be required for protein-protein interactions (Figure 1B). Sequencing of *At5g40820* from line 1-6 [13], hereafter referred to as *alt1-2*, revealed a substitution of T for C, resulting in the change of Leu to Phe at position 2553 in the predicted phosphatidylinositol 3- and 4-kinase domain of AtATR. Currently, it is not clear as to what the basis is for the dominant nature of these mutations, although it can be speculated that this may arise from the mutant versions of AtATR negatively affecting protein complexes in which AtATR participates, thereby compromising overall function of the complex with regard to detection of Al-dependent damage. The mutation in line 63-2 [13], which is also predicted to be an *alt1* allele based on map location, could not be identified although this mutant is physiologically identical to *alt1-1* and *alt1-2*.

Subsequently, *alt1-1* and *alt1-2* mutants, without the *als3-1* mutation in their respective backgrounds, were generated and analyzed for their growth in the presence of Al. As shown in Figure 1C, the *alt1-1* mutant roots have a profound increase in their capability to grow in an Al-toxic environment in comparison to both *als3-1* and Col-0 WT. Analysis of a previously characterized *atr* knockout mutant, *atr-2* [14–16], revealed that complete loss of activity of ATR results in a significant increase in Al tolerance compared to Col-0 WT, although this mutant is not comparable to *alt1-1* in terms of the magnitude of its Al tolerance. Consistent with this, analysis of the Al tolerance of *alt1-2* also revealed a substantial increase in Al tolerance compared to Col-0 WT (Figure S1 available online), suggesting that reduced function of AtATR may represent a viable strategy for increasing Al tolerance in crop plants.

Because *alt1-1* and *alt1-2* are dominant in nature, it was of interest to determine whether their root growth is affected similarly to *atr-2* after treatment with hydroxurea, which is a replication fork poison [14–16]. Treatment of *atr-2* with hydroxurea results in severe root-growth inhibition compared to Col-0 WT. As with *atr-2*, both *alt1-1* (Figure 1D) and *alt1-2* (Figure S2) demonstrated substantial reduction in root growth in the presence of 1 mM hydroxurea, suggesting that the toxic effects of Al are distinct from those of hydroxurea, especially in terms of the role of AtATR in the detection of each. These results indicate that, although dominant, *alt1-1* and *alt1-2* each represent partial loss-of-function mutants that likely reduce, but do not eliminate, the activity of AtATR, with these mutations possibly affecting only a subset of functions of AtATR rather than completely eliminating AtATR activity as in the *atr-2* mutant.

As previously described, the *alt1-1* and *alt1-2* mutations were identified based on their capability to suppress the Al hypersensitivity phenotype seen for *als3-1*, making it of interest to determine whether *atr-2* could also restore the growth of *als3-1* roots in an Al-toxic environment. It was found that whereas *als3-1* roots demonstrated severe root-growth inhibition in the presence of moderate levels of $AlCl_3$ in a soaked gel environment (pH 4.2), Al-treated roots of the *atr-2;als3-1* mutant were indistinguishable from Col-0 WT and *atr-2*

*Correspondence: paul.larsen@ucr.edu

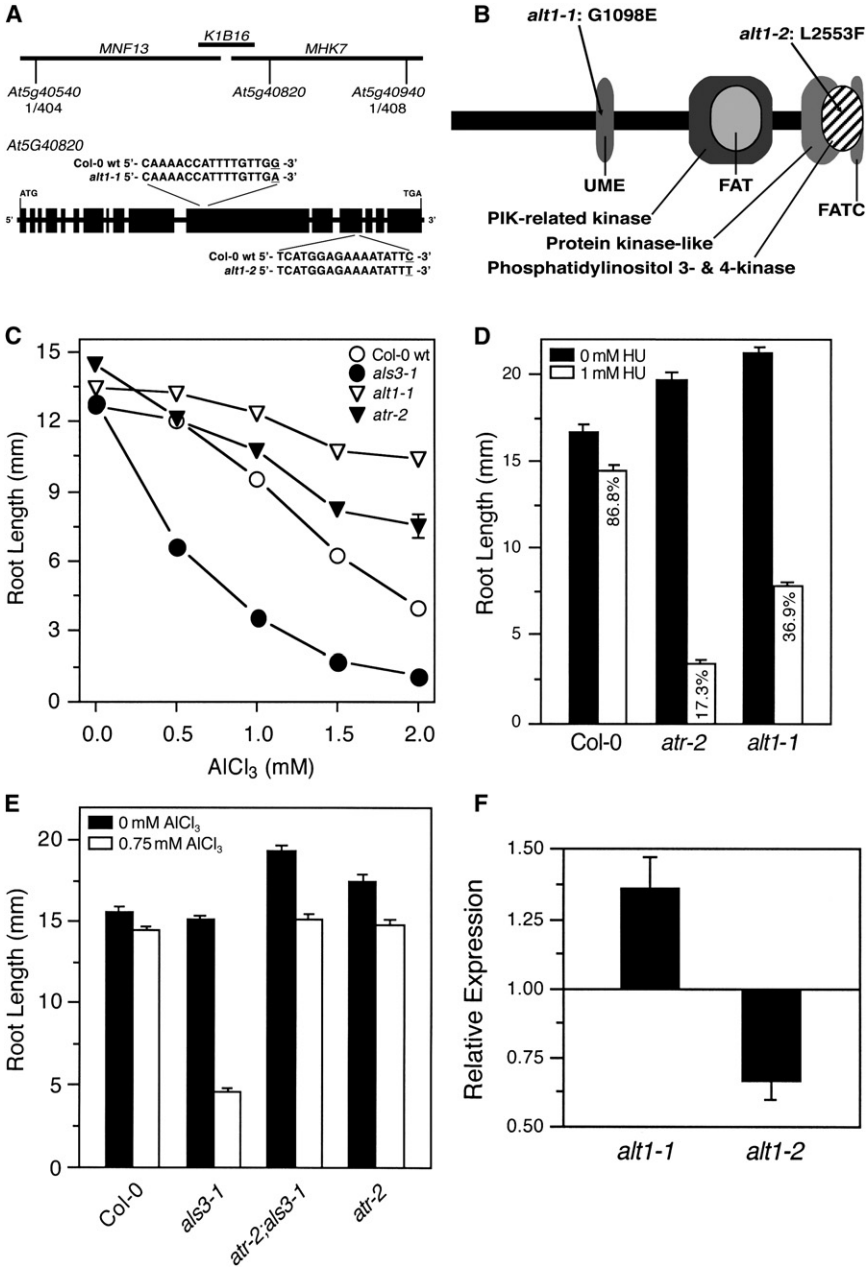

Figure 1. Isolation and Analysis of the *alt1* Mutations

(A) The *alt1-1* mutation was localized to chromosome 5 between the polymorphic markers *nga76* and *CIW9*. Sequencing of candidate genes amplified from *alt1-1* and *alt1-2* revealed mutations that result in amino acid substitutions in *At5g40820*, which encodes the AtATR factor that is required for detection and response to DNA damage.

(B) Schematic of the AtATR protein, showing predicted protein domains. The *alt1-1* mutation results in an amino acid substitution and consequent change in amino acid charge in the UME domain, which is predicted to be responsible for protein-protein interactions in known ATR homologs. The *alt1-2* mutation results in an amino acid substitution in a predicted phosphatidylinositol 3- and 4-kinase domain, with this mutation likely resulting in inappropriate steric hindrance because of introduction of a F adjacent to another F. In this schematic, FAT and FATC domains indicate conserved regions found in predicted PIK-related kinases.

(C) Analysis of root growth of the *alt1-1* mutant compared to Col-0 WT, *als3-1*, and *atr-2* in the presence of increasing concentrations of $AlCl_3$ in a soaked gel environment (pH 4.2) was performed. Whereas root growth of Al-treated Col-0 WT and especially *als3-1* were severely impacted after 7 days, both the *alt1-1*, without the *als3-1* mutation in its background, and the *atr-2* mutants displayed high levels of Al tolerance compared to Col-0 WT. Mean ± SE values were determined from 30 seedlings.

(D) *alt1-1* mutant roots are hypersensitive to the replication fork poison hydroxurea (HU). For this analysis, Col-0 WT, *alt1-1*, and *atr-2* seedlings grown hydroponically in the absence of HU for 4 days were subsequently exposed to either 0 mM or 1 mM HU for an additional 3 days, after which roots were measured. Mean ± SE values were determined from 30 seedlings.

(E) The *atr-2* mutation is capable of suppressing the severe Al hypersensitivity seen for *als3-1* mutant roots. For this analysis, an *atr-2;als3-1* double mutant was generated and analyzed in comparison to Col-0 WT, *als3-1*, and *atr-2* for its growth capability in the presence of 0.75 mM $AlCl_3$ in a soaked gel environment (pH 4.2). Mean ± SE values were determined from 30 seedlings.

(F) qPCR analysis was performed with primers that recognize the 3′-UTR of *AtATR* for Col-0 WT, *alt1-1*, and *alt1-2* seedlings to determine relative expression of *AtATR* in each. Only the *alt1-2* mutation was found to significantly reduce transcript levels of *AtATR*.

(Figure 1E), further indicating that, although dominant, *alt1-1* and *alt1-2* represent loss-of-function mutations of *AtATR*. A severe loss-of-function mutation affecting *AtATM* (*Ataxia Telangiectasia-Mutated*) [15–17], which is responsible for detecting double-strand DNA breakage in *Arabidopsis*, was not capable of suppressing the *als3-1* phenotype.

qPCR analysis was performed to determine whether either the *alt1-1* or *alt1-2* mutations affect the accumulation of *AtATR* transcript (Figure 1F). From this analysis it was found that only the *alt1-2* mutation has a significant negative impact on *AtATR* levels in mutant roots compared to Col-0 WT, suggesting that the *alt1-2* mutant phenotype may arise from a negative effect on protein function, transcript accumulation, or both. In contrast, it appears that the *alt1-1* mutation has no negative effect on transcript levels, suggesting that this mutation specifically reduces protein function, possibly through impaired interactions with partner proteins because of the *alt1-1* mutation being found in the UME domain.

Because there is an established relationship of the toxicity of many heavy metals with either promotion of DNA damage or inhibition of DNA repair [18–21], it was of interest to determine whether the *alt1* mutants demonstrated increased tolerance to a range of heavy metals. Although there was no obvious increase in tolerance of the *alt1* mutants to metals such as As(III), Cr(IV), Co(II), Se, and Pb(II), there were modest yet reproducible increases in the capability of *alt1-1* roots to grow in the

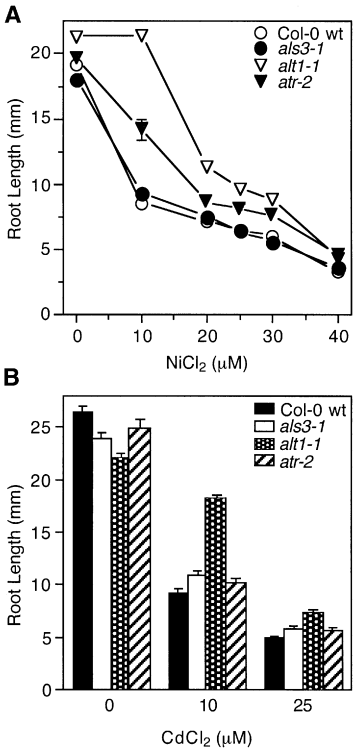

Figure 2. *alt1* Mutant Roots Are Resistant to Nickel and Cadmium

(A) *alt1-1* roots are tolerant to low levels of $NiCl_2$. Col-0 WT, *alt1-1*, *atr-2*, and *als3-1* seedlings were grown either in the absence or presence of increasing concentrations of $NiCl_2$ (pH 5.5) for 7 days after which root length was measured. Mean ± SE values were determined from 30 seedlings. *alt1-1* roots demonstrated a significant increase in root growth in the presence of levels of $NiCl_2$ that had a severe effect on Col-0 WT root growth. *als3-1* roots showed no difference in root growth compared to Col-0 WT, suggesting that *als3-1* is not generally sensitive to metal stress.

(B) *alt1-1* roots have a modest increase in tolerance to $CdCl_2$. Col-0 WT, *alt1-1*, *atr-2*, and *als3-1* were grown for 7 days in the absence or presence of 10 μM $CdCl_2$ (pH 5.5) after which root length was measured. Mean ± SE values were determined from 30 seedlings. *alt1-1* roots were found to be modestly tolerant to low levels of $CdCl_2$ in the medium. This is distinctly different from Col-0 WT, *als3-1*, and *atr-2*, all of which showed comparable levels of root-growth inhibition in the presence of $CdCl_2$. As with $NiCl_2$ treatment, the results with $CdCl_2$ indicate that the phenotype of the *als3-1* mutant is specific to Al.

presence of low levels of both $NiCl_2$ and $CdCl_2$ in a hydroponic environment (Figures 2A and 2B), suggesting that AtATR is also required for roots to respond to damage caused by these heavy metals. Interestingly, *atr-2* and *alt1-2* roots (Figures S3 and S4) did not demonstrate the same level of tolerance to these metals, suggesting that the effect of the *atl1-1* mutation is distinctly different from the complete loss-of-function allele *atr-2*.

It has previously been shown that AtATR is responsible for regulating progression of the cell cycle, with AtATR triggering G2 arrest after accumulation of DNA damage [14]. In order to monitor whether cells are locked at the G2 stage of the cell cycle after Al treatment, a previously described approach, in which the GUS reporter was fused to the *CycB1;1* promoter and a truncated CDS containing a predicted mitotic destruction box, was used [14, 22]. Normal progression through the cell cycle should result in few cells being observed in the G2 stage at any one time, whereas treatments that inhibit the cell cycle would be expected to cause a detectable increase in the number of cells trapped in the G2 stage, thus resulting in the inappropriate accumulation of GUS activity. For this analysis, the *CycB1;1::GUS* transgene was introgressed into both *als3-1* and *alt1-1*, with plants subsequently grown either in the absence or presence of increasing levels of Al, after which roots were stained for GUS activity. As shown in Figure 3A, in the absence of Al, there was only limited GUS activity seen for Col-0 WT, *als3-1*, and *alt1-1* roots, which is consistent with normal cell-cycle progression under non-stressful conditions because of proper turnover of *CyclinB1;1*. Addition of low levels of Al resulted in a dramatic increase in GUS activity in root tips of both Col-0 WT and *als3-1*, with no concomitant increase in those of *alt1-1*, suggesting that Al induces G2 arrest in both Col-0 and *als3-1* roots. Interestingly, addition of increasing levels of Al resulted in repositioning of the zone of differentiation in Col-0 roots much closer to the tip, with root hairs appearing just behind the area of cell division in Al-treated roots. This phenomenon was most pronounced in *als3-1* roots treated with moderate to high levels of Al, with these roots, which were completely inhibited with regard to growth, showing what appeared to be complete differentiation of the root tip coupled with tremendous swelling of cells in the primary root along with initiation of lateral roots in this region. Unlike Col-0 WT roots, *als3-1* roots showed little to no GUS activity in the presence of moderate to high levels of Al, most likely because of loss of cell-cycle activity in this region resulting from full differentiation of the root tip. Consistent with this argument, Evans blue staining, which is a vital stain used to determine the viability of cells, indicated that the Al-treated roots of Col-0 WT and *als3-1* were still alive and that stoppage of root growth was not due to death of the root cells, because Col-0 and *als3-1* roots did not stain the dark blue that is normally seen for nonviable tissues (Figure 3B). Interestingly, even with high levels of Al, *alt1-1* roots, which maintained root growth, did not display unusual levels of GUS activity. This indicates that cell division in *alt1-1* roots is not arrested at the G2 stage of the cell cycle after Al treatment, which is consistent with loss of AtATR activity [14]. Consequently, *alt1-1* roots continue to progress through normal cell division even though the cells of the root tip are likely compromised by Al toxicity, which suggests that Al tolerance in *alt1-1* roots arises from an inability to recognize and respond to Al-dependent DNA damage.

Clearly, based on the phenotype of Al-treated *als3-1* roots, it appears that treatment with high levels of Al forces complete differentiation of the root tip, with this differentiation likely resulting in stoppage of root growth because of loss of stem cells resulting from Al-dependent damage. In order to determine whether maintenance of the quiescent center (QC) is negatively affected after Al treatment, a GUS-based marker for the cells of the QC, QC46 [23], was introgressed into both *als3-1* and *alt1-1*. For this analysis, Col-0 WT, *als3-1*, and *alt1-1*, all of which carried the QC46 GUS-based promoter trap, were grown either in the absence or presence of highly toxic levels of $AlCl_3$ (pH 4.2), after which roots were stained for GUS activity. In the absence of Al, all three lines demonstrated normal staining of the QC, as evidenced by accumulation of blue color near the root tip (Figure 3C). In contrast, growth in the presence of toxic levels of Al resulted in complete loss of GUS activity in both Col-0 WT and *als3-1*, indicating that an irreparable consequence of Al toxicity is loss of the progenitor cells of the QC, thus resulting in loss of the necessary stem cells for maintenance of root growth. In contrast, *alt1-1*

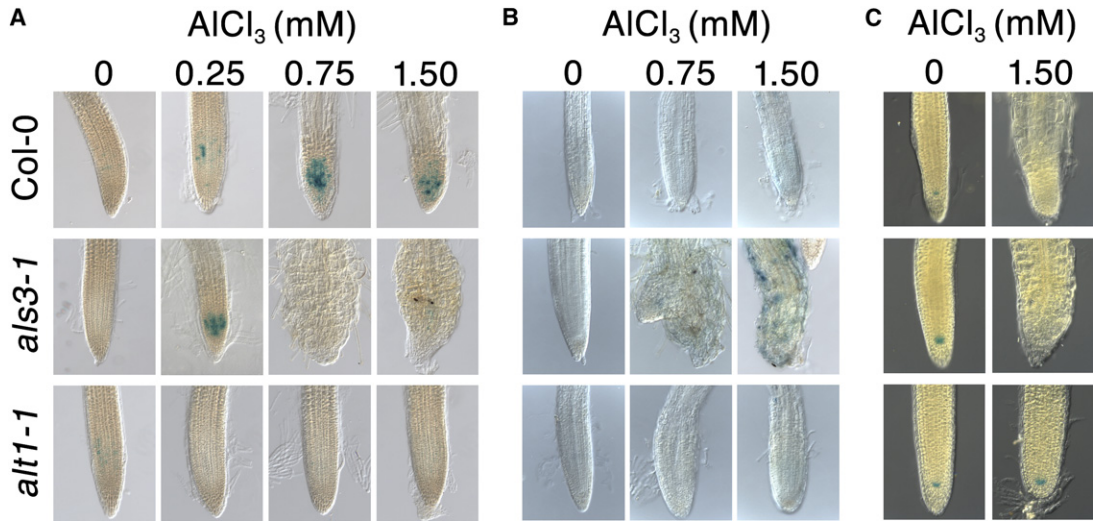

Figure 3. Al Prevents Cell-Cycle Progression and Quiescent Center Maintenance

(A) Growth in the presence of inhibitory levels of $AlCl_3$ results in a disproportionate increase in the number of cells trapped in the G2 phase of the cell cycle. A marker for cell-cycle progression, in which a truncated version of the CDS of *CyclinB1;1* including a predicted mitotic destruction box was fused to the *GUS* reporter gene, was introduced into both *als3-1* and *alt1-1*. For this analysis, Col-0 WT, *als3-1*, and *alt1-1* lines carrying the *CyclinB1;1::GUS* reporter were grown in the absence or presence of increasing concentrations of $AlCl_3$ in a soaked gel environment (pH 4.2), after which seedlings were stained for GUS activity. Growth in the absence of $AlCl_3$ resulted in minimal levels of GUS staining, indicating that few cells were captured in the G2 phase under these conditions. Addition of $AlCl_3$ to Col-0 WT and *als3-1* resulted in a profound increase in GUS staining, showing that Al treatment causes an increased number of cells to be trapped in the G2 phase of the cell cycle. Treatment with high levels of $AlCl_3$ resulted in complete loss of GUS activity in *als3-1* roots, indicating that cells in the root tip of *als3-1* were completely differentiated and not undergoing cell division. *alt1-1* roots showed only basal levels of GUS activity even with high $AlCl_3$, indicating that *alt1-1* mutant roots fail to arrest the cell cycle in response to Al because of reduced AtATR activity. More than 30 seedlings of each line were tested for this analysis.

(B) Evans blue staining indicates that although severely arrested, *als3-1* roots are viable after Al treatment. Col-0 WT, *als3-1*, and *alt1-1* seedlings were grown in the absence or presence of $AlCl_3$ in a soaked gel environment (pH 4.2) for 7 days and subsequently stained with Evans blue, which is a vital stain. Even after Al treatment, Evans blue staining was minimal in all samples tested indicating that Al-dependent root-growth inhibition does not result from tissue death. More than 30 seedlings of each line were tested for this analysis.

(C) Treatment with high levels of Al results in loss of the quiescent center (QC) in *Arabidopsis* roots. A GUS-based marker for the root quiescent center, QC46, was introduced into *als3-1* and *alt1-1*. Col-0 WT, *als3-1*, and *alt1-1* carrying the QC46 GUS promoter trap were grown either in the absence or presence of 1.50 mM $AlCl_3$ in a soaked gel environment (pH 4.2), after which the seedlings were stained for GUS activity. In the absence of Al treatment, all samples displayed normal staining at the position of the QC in the root tip. Treatment with levels of Al that result in severe root-growth inhibition caused loss of GUS activity in both Col-0 WT and *als3-1* roots, indicating that Al forces the QC in both of these lines to fully differentiate, with this loss of stem cells in the root tip likely being the major underlying cause in root-growth inhibition after Al treatment. In contrast, *alt1-1* roots displayed GUS activity at the position of the QC even after Al treatment, indicating that loss of AtATR function leads to failure to trigger differentiation of the QC in the presence of Al. More than 30 seedlings of each line were tested for this analysis.

roots displayed normal GUS activity in the QC even in the presence of highly toxic levels of Al. This indicates that *alt1* loss-of-function mutants are incapable of detecting Al-dependent damage and fail to trigger impaired cells of the QC to differentiate, with this failure likely being the basis for the increased capability for root growth in an Al-toxic environment seen for *alt1* mutants.

It is documented that one major consequence of Al toxicity is DNA damage in both animals and plants [24–27], possibly resulting from oxidative stress or inappropriate release and/or localization of DNase enzymes. Because ATR is known to be responsible for detecting and responding to DNA damage, Comet assays were performed for untreated and Al-treated roots in order to assess whether Al results in DNA fragmentation in *Arabidopsis* under these experimental conditions. As shown in Figure 4A, treatment with Al results in a modest yet reproducible increase in genomic DNA fragmentation for Col-0 WT, *als3-1*, and *alt1-1;als3-1*, which supports previous findings that Al negatively impacts DNA integrity, although it is not currently clear as to whether this represents the damage that AtATR is responsible for detecting after Al treatment. Interestingly, the *alt1-1* mutation does not actually prevent accumulation of DNA damage, thus indicating that the increased capability to grow in an Al-toxic environment seen for roots of this mutant arises from failure to recognize and respond to Al-dependent DNA damage.

Loss of ATR function has previously been shown to result in enhanced telomere shortening when telomere homeostasis is compromised [17]. Terminal restriction fragment (TRF) analysis, which consists of digestion of genomic DNA with an enzyme that cuts outside of the telomeric repeat region and subsequent Southern analysis with a radio-labelled probe that recognizes this region, was performed with genomic DNA isolated from Col-0 WT, *als3-1*, *alt1-1;als3-1*, and *tert* roots grown in the absence and presence of highly toxic levels of $AlCl_3$ in order to determine whether Al toxicity is related to enhanced telomere shortening. This analysis did not reveal any detectable increase in telomere degradation when comparing controls with the respective Al-treated samples or the *als3-1* and *alt1-1;als3-1* mutants with Col-0 WT, indicating that Al toxicity does not cause accelerated telomere degradation. In support of this, the *tert* mutant [28], which represents a loss-of-function mutation in *Arabidopsis* telomere reverse transcriptase and is compromised in its capability to maintain telomere length, did not show any evidence of enhanced telomere shortening after Al treatment.

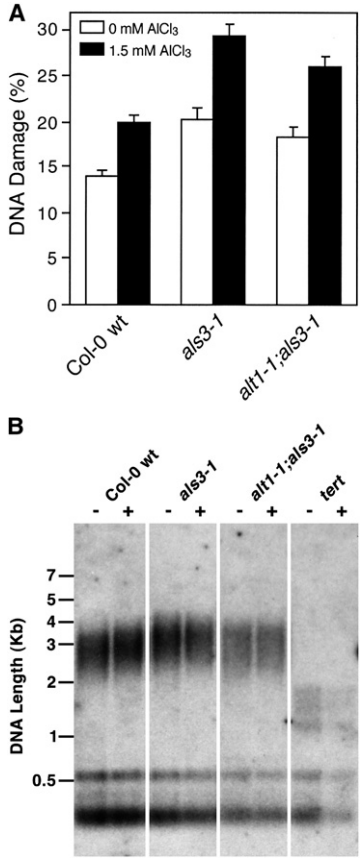

Figure 4. Al Toxicity Results in Increased DNA Damage in *Arabidopsis* Roots

(A) Comet assay analysis indicates that growth in an Al-toxic environment results in increased DNA fragmentation. Col-0 WT, *als3-1*, and *alt1-1;als3-1* seedlings were grown in the absence or presence of 1.50 mM $AlCl_3$ in a soaked gel environment (pH 4.2) for 7 days after which nuclei were isolated and analyzed with the Comet assay, which detects DNA fragmentation. In all samples tested, Al treatment resulted in a modest yet reproducible increase in DNA fragmentation indicating that Al directly affects DNA integrity. The *alt1-1* mutation does not prevent DNA damage, but rather fails to detect and/or respond to this damage as a result of loss of AtATR function.

(B) Al exposure is not correlated with enhanced telomere shortening. Col-0 WT, *als3-1*, *alt1-1;als3-1*, and *tert* seedlings were grown in the absence or presence of 1.50 mM $AlCl_3$ in a soaked gel environment (pH 4.2) for 7 days, after which genomic DNA was isolated, digested with Tru1I, electrophoretically separated, blotted, and hybridized with a telomere-specific DNA probe. In each line tested, Al treatment had no effect on telomere length, indicating that although AtATR is required for telomere maintenance, Al-dependent root-growth inhibition is not dependent on enhanced telomere shortening.

Although it cannot be ruled out that AtATR serves to detect Al-dependent damage that affects a currently undefined cellular component, it is likely (based on our results) that Al toxicity results in AtATR-monitored DNA damage, with this damage being unique from what has been previously attributed to AtATR as part of its assessment function for compromised DNA integrity. For example, treatment with either hydroxurea or ionizing radiation results in severe growth retardation of *atr* loss-of-function mutants compared to WT, which is opposite to the response of *atr* loss-of-function mutants to Al. Regardless of the mechanism by which Al causes DNA damage, it is clear from the response of the *atr* loss-of-function mutants that DNA damage is paramount with regard to Al-dependent inhibition of root growth. Apparently though, it is not necessarily the accumulation of DNA damage that is deleterious to root growth, but rather the detection of this damage by AtATR, which results in the active removal of impaired cells from the stem cell pool in the root tip and consequently loss of the QC. Although this appears to be a strategy that is imprudent for individual plants for growth and survival in Al-toxic soils, it is likely that this approach developed in order to prevent passage of compromised DNA to subsequent generations at the expense of the viability of individual plants challenged by this toxic environment. It is expected that, by removing or reducing the activity of this ATR-dependent self-assessment mechanism specifically at the root tip, it will be possible to engineer crop plants that are capable of surviving and possibly thriving in an Al-toxic environment, thereby increasing global crop productivity in regions that are challenged by the negative consequences of Al-toxic soils.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and four figures and can be found with this article online at http://www.current-biology.com/cgi/content/full/18/19/1495/DC1/.

Acknowledgments

We thank Anne Britt of the University of California-Davis and Lewis Feldman of the University of California-Berkeley for seed stocks, along with Patricia Springer of the University of California-Riverside for help with microscopy. This work was supported by the Functional and Regulatory Systems Cluster Program of the National Science Foundation (award number 0515482) and by a National Research Initiative Competitive Grant 2005-35100-16004 from the USDA Cooperative State Research, Education, and Extension Service.

Received: June 20, 2008
Revised: August 8, 2008
Accepted: August 12, 2008
Published online: October 2, 2008

References

1. von Uexkull, H.R., and Mutert, E. (1995). Global extent, development and economic impact of acid soils. Plant Soil *171*, 1–15.
2. Kochian, L.V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. *46*, 237–260.
3. Ma, J.F., Hiradate, S., and Matsumoto, H. (1998). High aluminum resistance in buckwheat. Plant Physiol. *117*, 753–759.
4. Delhaize, E., Ryan, P.R., and Randall, P.J. (1993). Aluminum tolerance in wheat (*Triticum aestivum* L.) II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol. *103*, 695–702.
5. Miyasaka, S.C., Buta, J.G., Howell, R.K., and Foy, C.D. (1991). Mechanism of aluminum tolerance in snapbeans. Root exudation of citric acid. Plant Physiol. *96*, 737–743.
6. Furukawa, J., Yamaji, N., Wang, H., Mitani, N., Murata, Y., Sato, K., Katsuhara, M., Takeda, K., and Ma, J.F. (2007). An aluminum-activated citrate transporter in barley. Plant Cell Physiol. *48*, 1081–1091.
7. Hoekenga, O.A., Maron, L.G., Piñeros, M.A., Cançado, G.M., Shaff, J., Kobayashi, Y., Ryan, P.R., Dong, B., Delhaize, E., Sasaki, T., et al. (2006). *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. Proc. Natl. Acad. Sci. USA *103*, 9738–9743.
8. Magalhaes, J.V., Liu, J., Guimarães, C.T., Lana, U.G., Alves, V.M., Wang, Y.H., Schaffert, R.E., Hoekenga, O.A., Piñeros, M.A., Shaff, J.E., et al. (2007). A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat. Genet. *39*, 1156–1161.
9. Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M., Ahn, S.J., Ryan, P.R., Delhaize, E., and Matsumoto, H. (2004). A wheat gene encoding an aluminum-activated malate transporter. Plant J. *37*, 645–653.

10. Larsen, P.B., Tai, C.Y., Kochian, L.V., and Howell, S.H. (1996). *Arabidopsis* mutants with increased sensitivity to aluminum. Plant Physiol. *110*, 743–751.
11. Larsen, P.B., Kochian, L.V., and Howell, S.H. (1997). Al inhibits both shoot development and root growth in *als3*, an al sensitive *Arabidopsis* mutant. Plant Physiol. *114*, 1207–1214.
12. Larsen, P.B., Geisler, M.J., Jones, C.A., Williams, K.M., and Cancel, J.D. (2005). *ALS3* encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J. *41*, 353–363.
13. Gabrielson, K.M., Cancel, J.D., Morua, L.F., and Larsen, P.B. (2006). Identification of dominant mutations that confer increased aluminium tolerance through mutagenesis of the Al-sensitive *Arabidopsis* mutant, *als3–1*. J. Exp. Bot. *57*, 943–951.
14. Culligan, K., Tissier, A., and Britt, A. (2004). ATR regulates a G2-phase cell-cycle checkpoint in *Arabidopsis thaliana*. Plant Cell *16*, 1091–1104.
15. Culligan, K.M., Robertson, C.E., Foreman, J., Doerner, P., and Britt, A.B. (2006). ATR and ATM play both distinct and additive roles in response to ionizing radiation. Plant J. *48*, 947–961.
16. Friesner, J.D., Liu, B., Culligan, B., and Britt, A.B. (2005). Ionizing radiation-dependent gamma-H2AX focus formation requires ataxia telangiectasia mutated and ataxia telangiectasia mutated and Rad3-related. Mol. Biol. Cell *16*, 2566–2576.
17. Vespa, L., Couvillion, M., Spangler, E., and Shippen, D.E. (2005). ATM and ATR make distinct contributions to chromosome end protection and the maintenance of telomeric DNA in *Arabidopsis*. Genes Dev. *19*, 2111–2115.
18. Kasprzak, K.S., Sunderman, F.W., Jr., and Salnikow, K. (2003). Nickel carcinogenesis. Mutat. Res. *533*, 67–97.
19. Waisberg, M., Joseph, P., Hale, B., and Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology *192*, 95–117.
20. Bertin, G., and Averbeck, D. (2006). Cadmium: Cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie *88*, 1549–1559.
21. Salnikow, K., and Zhitkovich, A. (2008). Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: Nickel, arsenic, and chromium. Chem. Res. Toxicol. *21*, 28–44.
22. Colon-Carmona, A., You, R., Haimovitch-Gal, T., and Doerner, P. (1999). Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J. *4*, 503–508.
23. Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. (2003). SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes Dev. *17*, 354–358.
24. Achary, V.M.M., Jena, S., Panda, K.K., and Panda, B.B. (2008). Aluminium-induced oxidative stress and DNA damage in root cells of *Allium cepa* L. Ecotoxicol. Environ. Saf. *70*, 300–310.
25. Banasik, A., Lankoff, A., Piskulak, A., Adamowska, K., Lisowska, H., and Wojcik, A. (2005). Aluminum-induced micronuclei and apoptosis in human peripheral-blood lymphocytes treated during different phases of the cell cycle. Environ. Toxicol. *20*, 402–406.
26. Lankoff, A., Banasik, A., Duma, A., Ochniak, E., Lisowska, H., Kuszewski, T., Góźdź, S., and Wojcik, A. (2006). A comet assay reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes. Toxicol. Lett. *161*, 27–36.
27. Lima, P.D., Leite, D.S., Vasconcellos, M.C., Cavalcanti, B.C., Santos, R.A., Costa-Lotufo, L.V., Pessoa, C., Moraes, M.O., and Burbano, R.R. (2007). Genotoxic effects of aluminum chloride in cultured human lymphocytes treated in different phases of cell cycle. Food Chem. Toxicol. *45*, 1154–1159.
28. Fitzgerald, M.S., Riha, K., Gao, F., Ren, S., McKnight, T.D., and Shippen, D.E. (1999). Disruption of the telomerase catalytic subunit gene from *Arabidopsis* inactivates telomerase and leads to a slow loss of telomeric DNA. Proc. Natl. Acad. Sci. USA *96*, 14813–14818.